SOIL TEMPERATURE AS A FACTOR AFFECTING THE PATHOGENICITY OF CORTICIUM VAGUM ON THE PEA AND THE BEAN [1]

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INTRODUCTION

The present paper is the third of a series of articles [2] dealing with the effects of soil temperature on the pathogenicity of *Corticium vagum* B and C. The two earlier publications *(13, 14)*[3] dealt primarily with the power of this organism to produce cankers on the stems of the Irish potato. In these publications results were presented from both field and greenhouse experiments which showed a definite and vital relation to exist between the temperature of the soil and the pathogenic action of *Corticium vagum* on this particular host. Such opportunity for further study of the parasitism of this organism was offered by the wide range of hosts on which the sterile or "Rhizoctonia" stage of *Corticium vagum* becomes parasitic, that studies similar to those on the potato were extended to include a number of additional hosts. The results of such studies on the pea and the bean, together with observations on the growth reaction of the fungus to temperature, are included in the following article.

APPARATUS AND METHODS

In the various pathogenicity studies the host plants were grown in metal cans 7 inches in diameter and 12 inches deep. These were submerged in a series of water jackets known at the University of Wisconsin as the Wisconsin soil-temperature tanks. These tanks, together with the methods for temperature and soil-moisture control, were described in the earlier work on the potato *(13)*. Such variations in the methods and operations as were therein employed will receive consideration in relation to the individual experiments.

Temperatures given in the various tables represent the mean temperatures at which the water was maintained in each of the different tanks. Fluctuations in these temperatures did not exceed in general more than one and one-half degrees from those given in the tables, even at the extreme high and low temperatures, and were not of more than a few hours' duration. Although the surface soil in the cans was kept approximately 2 inches below the surface of the water in the tanks, the soil

Journal of Agricultural Research,
Washington, D. C.

Vol. XXV, No. 11
Sept. 15, 1923
Key No. Utah—18

60096—23——1

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to a depth of 1 inch at the higher temperatures fell about 1° below that of the surrounding water. The reverse condition was found true for the soil at the lower temperatures of 9° and 12° C. This relation is of importance, as the greater amount of damage to the stems of both the pea and the bean occurred within the first 2 inches of surface soil.

In preliminary experiments, the fungus used was found to attack vigorously the tissues of the stems of both the pea and the bean. Under severe conditions, it proved to be able to attack, and even destroy, the plumule and the cotyledons of the young seedlings, and often severely damaged the secondary roots arising from the developing hypocotyl. The lesions produced on the stems were observed to vary in depth and area from mere browning and slight destruction of the epidermis, to extensive and ugly ulcers sufficiently deep practically to sever the stem. As in the earlier work with the potato, it was found that the number of stems showing injury gave no true index to the degree of damage caused by *Corticium vagum* on these plants at the various temperatures. With a view to obtaining a more accurate expression of this relation, all the diseased plants occurring at each of the various temperatures were divided into three separate classes: Slightly injured, severely injured, and cut off.[4] The percentage of plants found in these three classes were then multiplied by the units 1, 2, and 3, respectively. Quantitative values thus determined are included in the various tables under the caption, "Intensity of injury."

TEMPERATURE STUDIES WITH THE PEA

Prior to starting the temperature studies on the pea, the author had observed a series of experiments conducted by Dr. F. R. Jones with respect to the relation of soil temperature to the nature and type of lesion caused by *Corticium vagum* on this particular host. This work[5] consisted of growing five separate crops of peas in soil inoculated with the sterile stage of *Corticium vagum* at a depth of 1 inch and at temperatures of 9.5°, 12.2°, 15°, 18°, 20.5°, 23.5°, 26°, and 28° C. The first four of these experiments are interesting primarily in the fact that the steam-sterilized soil, regardless of the manner of inoculation, gave results which were so exaggerated in severity as to render them valueless for obtaining reliable temperature data. The seedlings, Jones states, were so promptly and vigorously attacked that only at the extreme high and low temperatures did the plants succeed in getting through the soil.

In growing the fifth crop Jones used unsterilized pasture soil, proved, by preliminary tests, to be free from parasitic strains of *Corticium vagum*. This he inoculated with one-fifth its weight of the infected soil used in the previous tests. Four cans for the growth of peas were maintained at each of the eight temperatures indicated in the table. Two of the cans, at each temperature, containing inoculated soil were planted with seven peas each; a third can with inoculated soil was planted with an equal number of cotton seeds; the fourth can, containing uninoculated soil,

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[4] In the first group, *slightly injured*, were placed all plants showing distinct lesions but not damaged to such an extent as to interfere with continued growth. In the second class, *severely injured*, were included such plants as showed injury severe enough to definitely injure the plant in its subsequent growth. In the group, *cut off*, were included all plants with plumules destroyed and those with stems severed by the fungus subsequent to infection.

[5] The results of these experiments were compiled by Dr. Jones in the form of a report now on file in the department of plant pathology at the University of Wisconsin and are summarized here with his approval. The additional work of the author with the pea as presented here must be considered as a continuation of that begun by Dr. Jones.

was planted with seven peas and two cotton seeds and held as a control.
The results as reported are graphically shown in figure 1 and in Table I.
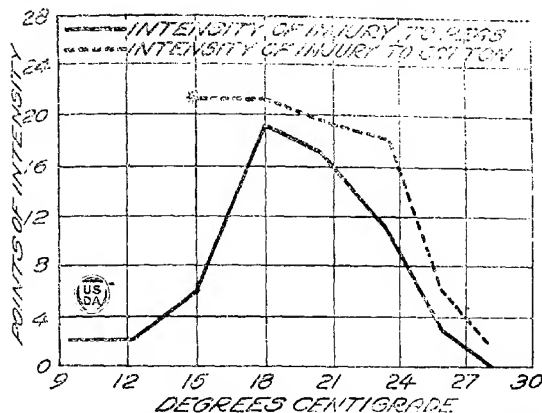All control plants were reported to be free from lesions.

Fig. 1.—Effect of soil temperature upon the severity of injury to peas and cotton caused by *Corticium vagum*.

TABLE I.—*Effects of growing pea and cotton plants at various temperatures in soil inoculated with Corticium vagum*

PEAS

Temperature at depth of 1 inch.	Plants grown in uninoculated soil.	Plants grown in inoculated soil.					
		Total number.	Number slightly injured.	Number severely injured.	Number cut off.	Number uninjured.	Total intensity of injury.[a]
°C.							
9.5	3	11	2				0	2
12.2	7	13		1		12	2
15.0	6	10	2	2		6	6
18.0	6	11	2	4	3	2	19
20.5	6	12	2	3	3	4	17
23.5	7	11		4	1	7	11
26.0	7	11	1	1		9	3
28.0	7	13				13	

COTTON

15.0	1						21
18.0	2						21
21.5	2	2		2			19
23.5	2	3		3			18
26.0	2	7	4	1		2	6
28.0	2	7	2			5	2

[a] In estimating the extent of injury upon the peas Jones used the following scale of values:
"Digit 1 represents a lesion on the stem which may not greatly damage the plant in its subsequent growth.
"Digit 2 represents a lesion on the stem which appears to be severe enough to greatly injure the plant in subsequent growth.
"Digit 3 represents destruction of the shoot, the root alone having made growth.
"The sum of the digits representing all the injuries found at any temperature is taken to represent the total intensity of injury at that temperature.
"In estimating the intensity of injury to cotton, the same scale was used, except that the digit 3 represents the destruction of the seed before germination. Thus the total number of seeds planted multiplied by 3 represents the total destruction of plants."

From the series of experiments Jones drew the following "tentative" conclusions:

1. The use of steam-sterilized soil inoculated with cultures of Rhizoctonia was found to exaggerate very greatly the amount of injury which the fungus causes to appear.
2. Rhizoctonia appears to do the greatest amount of injury to peas when the soil temperatures lie between 12° and 27° C. with a maximum amount of injury near 18° C.
3. Within the limits tried neither high nor low temperatures completely inhibited injury from Rhizoctonia.
4. Lesions produced by Rhizoctonia upon peas appear to be characteristic, resembling those upon potatoes.
5. It is frequently very difficult to reisolate Rhizoctonia from peas because of the presence of rapidly growing secondary invaders.
6. In a single comparison of the pathogenicity of a strain of Rhizoctonia from peas and one from potatoes no difference was observed in the nature or the intensity of the injury done.
7. Injury by Rhizoctonia to peas may be classified as follows:
   A. Destruction of the entire embryo of the germinating seed.
   B. Destruction of the primary shoot which may later be replaced by one or more secondary shoots.
   C. Production of lesions of greater or less intensity upon the stem below the surface of the ground.
   D. The early destruction of the cotyledons, thus depriving the young plant of its stored food.

In the experiments on the pea conducted by the author three successive crops were grown in the "tanks" at soil temperatures varying from 9° to 29° C.

EXPERIMENT 1.—In this experiment peas were grown at the various temperatures in unsterilized pasture soil inoculated with *Corticium vagum*[a] five days previous to planting. One can in each temperature tank was used for growing the crop in the inoculated soil. A similar series with uninoculated soil was arranged as a control. Ten seeds were planted in each can of the two series, and 2 days later the tanks were adjusted to their various temperatures. The results obtained from the inoculated soil 19 days after planting are recorded in Table II. All control plants were found to be free from lesions.

TABLE II.—*Effect of growing peas at various temperatures in soil inoculated with Corticium vagum (Experiment 1)*

Temperature at depth of 1 inch.	Number of seeds planted.	Number of plants grown in uninoculated soil.	Plants grown in inoculated soil.						
			Total number.	Number slightly injured.	Number severely injured.	Number cut off.	Number uninjured.	Percentage injured.	Intensity of injury (points).
°C.									
9.3	10	9	7	1	....	....	6	14.2	14.2
11.7	10	10	8	4	1	....	3	62.5	75.0
15.0	10	10	9	4	1	1	3	66.2	100.0
18.0	10	9	8	3	3	1	1	87.5	150.0
20.8	10	10	10	1	2	1	6	40.0	80.0
23.5	10	10	10	1	2	....	7	30.0	50.0
26.6	10	10	10	....	....	....	10	....	....
29.0	10	10	10	....	....	....	10	....	....

The results are especially interesting in showing the inhibiting effect of the high and low temperatures on the pathogenic action of the fungus. No damage occurred to the plants at 26° and 29° C., and plants at 9°

[a] This particular strain of *Corticium vagum* was provided by Dr. F. R. Jones, who had previously isolated it from diseased peas obtained in Wisconsin. The soil in the cans in Experiments 1 and 2 was inoculated with small quantities of soil previously inoculated for this purpose with this specific organism.

were but slightly injured. However, severe injury occurred between the temperatures of 11° and 23°. Growing-point destruction resulted only at 15°, 18°, and 21°.

EXPERIMENT 2.—In Experiment 2 plants were grown at the various temperatures in unsterilized soil inoculated with the same strain of *Corticium vagum* as that used in Experiment 1. These were checked by a similar number of plants grown in uninoculated soil. Except for the increased number of seeds planted in each pot, Experiment 2 was practically a duplicate of Experiment 1. Twelve seeds were planted in each pot in both the inoculated and uninoculated soil. The results were as shown in Table III.
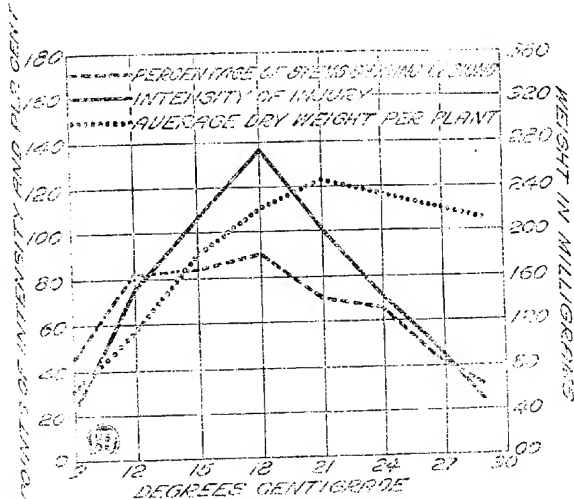

Fig. 2.—Effect of soil temperature upon the severity of stem injury caused by *Corticium vagum* and upon the growth of the pea plant as determined by the dry weight. The curve showing dry weight represents the average of the results from Experiments 1 and 2.

TABLE III.—*Effect of growing peas at various temperatures in unsterilized soil inoculated with Corticium vagum (Experiment 2).*

Temperature at depth of 1 inch.	Number of seeds planted.	Number of plants grown in uninoculated soil.	Plants grown in inoculated soil.							
			Total number.	Number very slightly injured.	Number slightly injured.	Number severely injured.	Number cut off.	Number uninjured.	Percentage injured.	Intensity of injury (points).
° C										
9.2	12	10	12	9	....	....		3	75	37.5
12.7	12	11	10	5	5	......			100	75.0
15.0	12	12	10	4	3	3	1	2	91	110.0
18.0	12	12	12	4	2	4	....		100	124.3
21.0	12	12	9	2	4	3	....		100	122.1
24.0	12	12	10	3	5	2	1	1	99	105.0
26.5	12	11	11	4	3	3	....	4	66	99.7
29.0	12	12	12	6	1	1				49.9

Twenty-six days after planting, lesions were found on plants throughout the entire temperature range. Lesions which occurred at the extreme temperatures,[7] however, were very slight and in many cases consisted of little more than distinct browning of the outer tissues. The most severe destruction of the stem tissues occurred at the temperatures from 15° to 24° C. As in Experiment 1, few plumules or growing points were destroyed. The control plants were again found free from lesions.

TABLE IV.—*Average of results from pea Experiments 1 and 2, including dry weight of plants grown as controls in Experiment 3 (see figure 2)*

Average temperature.	Average percentage of plants injured.	Average total intensity of injury.	Average dry weight per plant
°C			Gm.
9.2	44.6	25.8	0.062
12.0	81.3	75.0	.112
15.0	83.3	105.0	.170
18.1	89.5	136.6	.222
21.0	70.0	101.0	.243
24.0	65.0	71.0	.235
26.6	45.0	50.0	.226
29.0	33.0	24.0	.216

a The figures represent the average of 12 plants on which determinations were made. The exaggerated severity of the damage occurring in the sterilized soil (Experiment 3) justified the inclusion of data on growth of the host plant with the more normal results of Experiments 1 and 2. (See Experiment 3.)

EXPERIMENT 3.—Greenhouse soil used in this experiment had been sterilized for two hours at 15 pounds' pressure and afterwards allowed to stand exposed to the atmosphere for three days before being placed in the cans. A small quantity of agar culture of *Corticium vagum* was then thoroughly mixed with the first 4 inches of earth in each can, and the seeds planted immediately thereafter at a depth of 2 inches.

As in the previous experiments, one can at each temperature was used for the growth of plants in the inoculated soil and a similar series was used as a control. After 16 days' growth, the plants in both series were photographed (Pl. 1). The control plants were then washed free from soil and the average dry weight of the plants determined for each temperature (Table IV and fig. 2). Observations on the diseased plants taken at the time are recorded in Table V and are shown graphically in figure 3.

TABLE V.—*Effects of growing peas at various temperatures in steam-sterilized soil infested with Corticium vagum (Experiment 3)*

Temperature at depth of 1 inch.	Number of seeds planted.	Number of plants grown in uninoculated soil.	Plants grown in inoculated soil.						
			Total number.	Number slightly injured.	Number severely injured.	Number cut off.	Number uninjured.	Percentage injured.	Intensity of injury (points).
°C.									
9.0	20	18	15			14	1	93.3	138.3
12.0	20	20	14		1	11	2	85.7	114.2
15.2	20	20	14			14		100.0	150.0
18.0	20	20	15			15		100.0	150.0
20.6	20	18	13		2	8	3	77.0	107.6
24.0	20	19							
26.4	20	18	14	1	3	3	6	57.1	64.0
28.0	20	20	16	2	2	3	9	43.7	50.7

[7] The number of plants showing slight injury justified separating the group *slightly injured* into two classes—*very slightly injured* and *slightly injured*. Each per cent in the former class was allowed one-half point, while the latter class was allowed its former rating of one point (see page 432, paragraph 1).

Under these severe conditions total destruction of the plumule resulted at temperatures of 15° and 18° C. At 9°, while most of the seed germinated normally, only 1 plant out a possible 29 escaped injury. At the higher temperatures the severity of attack decreased more rapidly than at temperatures below those most favorable for tissue destruction. Severe injury, nevertheless, was obtained at 28°. An interesting feature of the experiments appeared in the fact that damage to the primary roots occurred at temperatures of from 15° to 21°, although no visible lesions were found on the smaller fibrous roots of the plant.
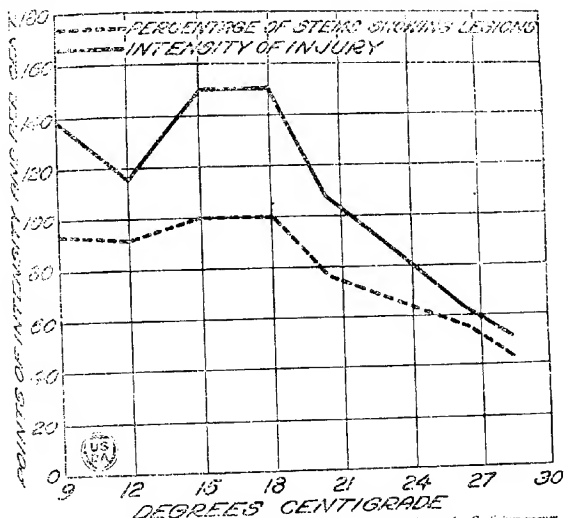

Fig. 3.—Effect of soil temperature upon the severity of injury caused to the pea by *Corticium vagum*.

DISCUSSION OF RESULTS OBTAINED WITH THE PEA

Results obtained from the three experiments on the pea agree essentially with those obtained by Jones in showing that within the temperature limits employed (9° to 29° C.) neither the high nor the low temperature entirely inhibited injury from *Corticium vagum*. However, in unsterilized soil, as in Experiments 1 and 2, the fungus is shown to be definitely limited in its pathogenic power at both the high and low temperatures. In these two experiments severe damage to the young plants was confined largely to temperatures of from 15° to 24°, with an optimum of approximately 18° C. Such favorable conditions for the pathogenicity of the fungus produced by sterilized soil, on the other hand, result in a much wider temperature range for the vigorous action of the fungus, although even under these conditions the most severe tissue destruction ranged between 12° and 24°. An equally definite optimum under these conditions was found at 18°.

In the earlier work on the potato (*13*), injury to the growing point of the young potato shoots appeared as a definite function of the rate at

which the young sprouts pushed through the soil in response to the temperature of the soil. With the pea, in the unsterilized soil of Experiments 1 and 2, the plumules were destroyed only at temperatures approximating the apparent optimum for tissue destruction (15° to 21° C.). In Experiment 3, however, under conditions more favorable for the pathogenic action of the fungus, growing-point injury occurred at 28°. Nevertheless, even under these severe conditions such injury was limited primarily to temperatures below 20°. As in the case of the potato, it appears quite probable that the rapid growth after germination played an important part in the protection of the plumules at the higher temperatures.

Normal pea growth was obtained throughout the entire range of temperatures of from 9° to 29° C. (Pl. 1, A). However, the most rapid germination and early growth, indicated by the time at which the plants appeared through the soil, occurred at 28° and 29°.[8] These results agree essentially with the optimum secured by Leitch (*10*). Dry-weight determinations showed the greatest growth of the normal plant for a period of sixteen days at 21° C. (Fig. 2 and Table IV.) Were the dry weights taken as an index to the temperature values at this period of the plant growth, it is clearly evident that the optimum for germination and early growth and the optimum for the later development of the plant would be widely different. This same relation was shown to exist for the potato under similar experimental conditions (*13*).

TEMPERATURE STUDIES WITH THE BEAN

Studies with the pea and the potato show clearly a similar soil temperature range for the pathogenic action of *Corticium vagum* on these two hosts. With both these plants 18° C. appeared optimum for tissue destruction. It will be recalled further that 18° approximates closely the soil temperatures found most favorable for the later and continued growth of both the pea and the potato. Whether this temperature relation between parasitism and host development is merely coincident with the two plants, due possibly to their similar temperature requirements for growth, or whether it is a condition determined primarily by a fixed character of the pathogen, does not appear clearly from the data obtained. Such data as Jones has supplied on cotton (Table I, fig. 1) support the latter possibility. However, to settle this question, additional experiments with other plants having temperature requirements different from either the pea or the potato appeared necessary. The work and observations of Reynolds (*12*), Reddick (*11*), Barrus (*4*), and Burkholder (*6*) on the relation of temperature to the growth of the bean suggested this plant as a favorable host for this additional study. Two experiments were accordingly made with this host.

EXPERIMENT 1.—Seven temperatures were employed, as indicated in Table VI. The soil used had been steam-sterilized and then inoculated with *Corticium vagum*[9] four weeks prior to the planting of the beans, and had grown during this period two crops of cress and radish seedlings. Before the beans were planted, however, the inoculated soil in the various cans was emptied and thoroughly mixed. Three cans were then filled with the inoculated soil at each temperature, and fifteen seeds[10] planted

, inches deep in each can. Uninoculated soil previously used to check cress and radish experiments was similarly mixed and used for growing control bean plants.

Plantings were made on April 30 and the temperatures immediately adjusted. Seventeen days after the planting, on May 16, photographs were taken of the control plants and of one series of plants grown in the inoculated soil (Pl. 2, A and B). The data taken at this same date on plants grown in inoculated soil are shown graphically in figure 4 and in Table VI.
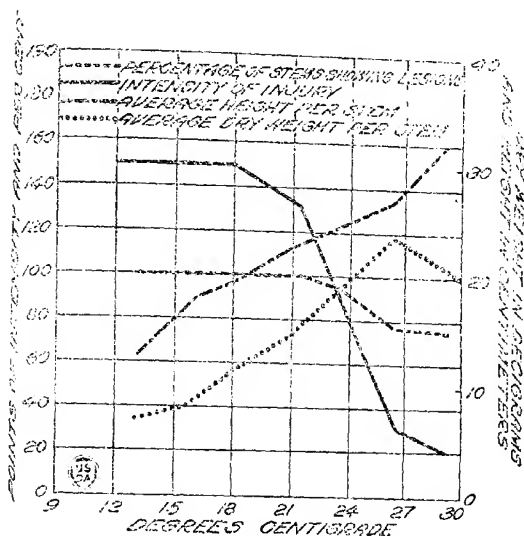

Fig. 4. —Effect of soil temperature on the pathogenic action of *Corticium vagum* on the bean. No plant survived at temperatures below 21° C. (see Pl. 2). The growth curves show the effects of soil temperatures upon host development, judged both from dry weight and height of plants.

TABLE VI.—*Effects of soil temperature upon the pathogenic action of Corticium vagum on the bean and also upon the development of the bean plant (Experiment 1)*

			Plants grown in inoculated soil.									
Temperature at depth of 1 inch.	Number of seeds planted.	Number of seeds grown in uninoculated soil.	Total number.	Number slightly injured.	Number slightly injured.	Number very severely injured.	Number cut off.	Number uninjured.	Percentage uninjured.	Intensity of injury.	Average height of plant.	Average dry weight of healthy plants 16 days old.
°C.											Cm.	Gm.
+52	45	14	25	Plumule	Destroyed					100.0	300.0	12.19	0.073
18.0	45	15	32	"	"					100.0	300.0	17.78	.087
18.5	45	13	29	"	"					100.0	300.0	18.79	.118
11.5	45	13	36			4	5	27		100.0	263.7	22.8	.140
23.5	45	13	38	1		13	4	18	9	94.7	188.3	24.1	.186
26.5	45	15	42	23		5	3	1	10	76.2	60.8	27.0	.235
29.5	45	15	43	30		2			11	74.4	40.0	32.0	.313

Lesions occurred on most of the plants throughout the entire temperature range. However, at 29° C. only two plants showed any definite tissue destruction. Such lesions as were observed on the remaining twenty plants at this temperature consisted of but slight browning of the outer stem tissue. The degree of damage was much increased at 27°, and at 24° a severe decrease in crop resulted. Though not expressed in the table or the curves, the maximum degree of injury occurred, without doubt, at temperatures of 18° and 21°. At these temperatures the plumules of the embryos were entirely destroyed and the hypocotyl of the seedlings were in many cases decayed. Although no plants appeared above the surface of the soil at 13° and 15°, the tissue destruction of the hypocotyl, which had made considerable growth at these temperatures, was not as great as at 18° and 21°.

EXPERIMENT 2.—As is evident from results presented, the conditions under which the plants were grown in Experiment 1 were so severe as to allow of no accurate quantitative expression of the degree of damage in the soil at and below 21° C. In an attempt to reduce the severity of these conditions, and in order to secure a more definite idea of the effects of the fungus on the bean at these lower temperatures, the inoculated and uninoculated soil used for Experiment 1 was emptied into separate piles, each diluted with twice its weight of unsterilized garden soil, and separately mixed. Sixteen cans were then refilled and temperatures adjusted. Fifteen seeds were again planted 2 inches deep in each of the 16 cans.

At the time the data were taken, 16 days after planting, all plants were above ground, except those grown at 9.4° C. However, these were well germinated. At 15° and 18° the plants in the inoculated soil appeared somewhat irregular in height, but otherwise they could not be distinguished from those grown in the uninoculated soil. The results of the examination of the underground parts are recorded in Table VII and are shown graphically in figure 5.

Under these less severe conditions, the damage to the stems was limited to the soil temperatures below 26.5° C. Severe injury occurred only in the soil held at 12°, 15°, 18°, and 21°, while the plumule destruction was noted only at 12° and 15°. The highest percentage of stems showing lesions, together with the most severe type of tissue destruction, was found at 18°, although because of injury to the growing points, a greater total intensity of injury is shown at 15°.

TABLE VII.—*Effects of soil temperature on the pathogenic action of Corticium vagum on the bean (Experiment 2)*

Temperature at depth of 1 inch.	Number of seeds planted.	Number of plants grown in uninoculated soil.	Plants grown in inoculated soil.							Average dry weight of healthy plants.
			Total number.	Number slightly injured.	Number severely injured.	Number cut off.	Number uninjured.	Percentage injured.	Intensity of injury.	
°C.										Gm.
9. 4........	20	15	16	4	....	......	11	25. 0	25. 0	0. 025
12. 5.......	20	18	18	8	1	1	8	55. 5	72. 0	. 066
15. 0.......	20	17	15	6	1	3	5	66. 6	113. 2	. 101
18. 0.......	20	18	16	8	3	......	5	68. 45	106. 1	. 131
21. 0.......	20	18	18	5	1	......	12	33. 3	38. 7	. 143
23. 6.......	20	16	19	5	....	......	14	26. 32	26. 32	. 190
26. 5.......	20	19	19	....	....	......	19	....	....	. 200
29. 7.......	20	19	19	....	....	......	19	....	....	. 206

DISCUSSION OF RESULTS OBTAINED WITH THE BEAN

In steam-sterilized soil *Corticium vagum* is shown to have a wide pathogenic range on the bean of from 9.4° to 29° C. With unsterilized soil, however, the fungus appeared definitely limited in its parasitic action under both the higher and lower temperatures tried in the experiments. In fact, under these more natural conditions, serious damage was confined entirely to the temperatures of 12.5°, 15°, 18°, and 21°, with a definite optimum for tissue destruction between 15° and 18. As with the pea and the potato, it is conceivable that the optimum might vary, contingent upon the conditions of the experiment, anywhere between 15° and 21°.
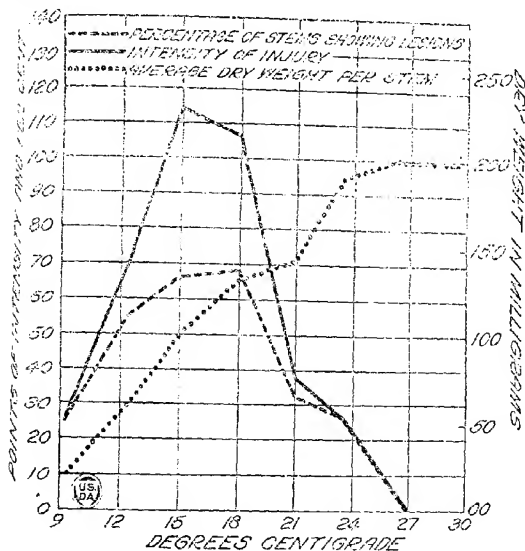

Fig. 5.—Effect of soil temperature upon the pathogenicity of *Corticium vagum* on the bean and upon the development of the host in uninoculated soil. The optimum temperature for the bean at the end of seventeen days lies clearly beyond the temperature range for the serious pathogenic action of the fungus.

General cultural experience shows the bean to be a plant which requires a high temperature for maximum growth and yield. Reddick (11) obtained the best growth at 28° C., while for germination Reynolds (12) found 30° to be the most favorable. Height measurements and dry-weight determinations in the present experiments, although not extensive, would indicate that the optimum soil temperature for the continued growth up to approximately three weeks, lies between 28° and 31°. The plants came up in all cases at the highest temperatures employed.

The parasitic relation of *Corticium vagum* on the bean is especially interesting in that the temperature most favorable for the growth of

the host falls completely outside of the dangerous pathogenic range of the parasite, and at such a high point as would allow the bean plants at their most favorable temperatures to escape practically uninjured. Jones' work (Table I, fig. 1) indicates that this same temperature relation holds equally true for the cotton plant. This agrees also with the field observations of Balls (*2*).

TEMPERATURE STUDIES WITH THE FUNGUS

Temperature requirements for the growth of *Corticium vagum* in pure culture have been determined with considerable definiteness by various workers. Rolfs (*15*) found 75° F. (24° C.) to be the most favorable for growth of the fungus. Hartman[11] obtained "no growth at 2°, slight growth at 8°, fair growth at 13° to 18°, profuse growth at 24° to 25°, fair growth at 30°, and no growth at 37° C." The fungus is shown by him to have a growth range of from 4° to 32° with an optimum at 24° to 25°. Balls (*3*) gives 5° to 32° as the growth range for the fungus and 23° as an optimum for continued growth. At 32°, he states, the fungus grows rapidly for two hours and then suddenly stops growth altogether. Balls (*3*) further reports a thermal death point of 50° for the young sclerotial cells when exposed at this temperature for a period of two minutes. He shows also that the optimum temperature for the growth of the fungus is a variable factor, definitely dependent upon the physiological history of the fungus as well as upon the immediate conditions under which the fungus is growing.

The wide temperature range through which *Corticium vagum* was found to destroy living plant tissue made it desirable to determine more accurately the temperature limits of the vital activities of the fungus, and to discover if possible its optimum temperature requirements for growth under varying conditions in pure culture. This latter relation is especially desired in view of the the low optimum of 18° C. found by the writer for the pathogenic activities of the parasite on the various hosts. The pressure of other duties, up to the present, has limited the work to the ordinary petri-dish method of study which, owing to the rapid growth of the mycelium, so shortens the period of observation as to lessen seriously the value of the results in interpreting the temperature reactions of the fungus. The data so far obtained, however, are of sufficient importance in their relation to the general problem to justify including them at this point.

In these studies small squares of a rapidly growing culture of the fungus, grown at 25° C., were placed in the center of the medium in each of the desired number of petri dishes. All the plates were then wrapped in sterile paper and kept at exactly the same temperature for 12 hours. At the end of this time the colonies were measured and the cultures placed directly in the incubators, which were held at the desired temperatures. Observations and measurements were made at regular intervals of 24 hours until the fungus had overgrown the media in the petri dishes. This, at the most favorable temperatures, occurred in about four days from the time that the temperatures were adjusted. Three series of the triplicate cultures were run at different temperatures. The average rates of growth obtained in series 1 and 2 are given in Table VIII and are shown graphically in figure 6.

The results thus obtained agree essentially with those of previous workers. Growth occurred through a range of temperatures of from 10° to 32.5° C., with an optimum both for linear and aerial mycelial growth for 96 hours of between 25° and 27°. It will be noted from the data tabulated, however, that at the higher temperatures the initial growth rate was not maintained; a progressive decrease with time was noted at all the different temperatures between 23.6° and 32.5°, inclusive, and only at the temperature of 22.4° was the rate of growth for the first 24 hours maintained for the entire period of observation.
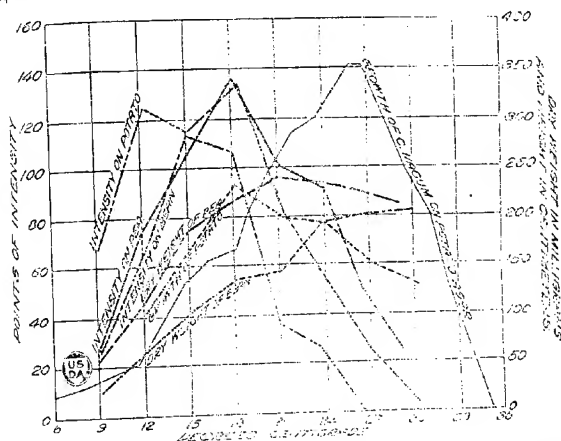

FIG. 6.—Showing the temperature range for the pathogenic action of *Corticium vagum* on the three hosts – pea, bean, and potato—in comparison with the temperature requirement for the growth of these three host plants (*13, tables on p. 465–472 and 475*) and for the growth of the fungus in pure culture.

TABLE VIII.—*Effect of temperature upon the growth of Corticium vagum on potato agar*

	Increased growth in diameter of colony for consecutive periods of 24 hours at different temperatures (°C).																
	4.6	8.2	10.9	14.6	16.3	18.1	19	20.1	22.4	23.6	25.7	26.6	29.8	31.2	32.6	35
Age of colony (hours):	Cm.	Cm.	Cm.	Cm.	Cm.	Cm.	Cm.	Cm.	Cm.	Cm.	Cm.	Cm.	Cm.	Cm.	Cm.	Cm.
24	0. 0	0. 0	3. 0	13. 5	16. 0	17. 0	18. 0	22. 5	28. 5	33. 0	38. 0	39. 0	36. 0	24. 0	20. 0	0
48	3. 0	3. 0	9. 5	16. 5	18. 0	18. 0	22. 0	25. 0	31. 0	29. 0	37. 0	38. 0	26. 0	22. 0	15. 0	0
72	1. 5	3. 5	6. 0	7. 5	16. 0	13. 5	22. 0	25. 0	25. 0	29. 0	36. 0	34. 0	23. 0	19. 0	10. 0	0
96	1. 0	4. 5	5. 0	15. 0	12. 5	....	30. 0	24. 0	29. 0	29. 0	30. 0	29. 0	15. 0	16. 0	7. 0	0
Average increase in diameter of colony	1. 5	2. 7	5. 6	13. 0	15. 6	16. 3	20. 5	24. 1	28. 3	30. 0	35. 2	35. 2	25. 0	20. 2	13. 1	0
Average linear growth of mycelium	.75	1. 35	2. 87	6. 5	7. 8	8. 1	10. 25	12. 1	14. 1	15. 0	17. 6	17. 6	12. 5	10. 1	6. 55	0

This decided falling off in the rate of growth with time at the higher temperatures is especially significant, in view of the low temperature of 23° C. which was found by Balls (3) to be optimum for continued growth of the fungus. This would indicate that had the experiment continued, a much lower optimum temperature than that found for the 96 hours

would have been shown. In fact, a study of the growth reactions of the fungus at temperatures of 23.6°, 25.7°, and 26.7° would suggest a possible optimum of 23.6° at the end of 120 hours. Balls noted the effect of the time element in his studies on the sterile stage of *Corticium vagum*, or "sore-shin" fungus, and studied the phenomenon in detail. He showed that the stopping point for the apical growth of the hyphae was lowered with increase of time. Again, this author points out that the "time element" varies with the different media on which the fungus is grown. On hard agar, he states, the growth was considerably inhibited by high temperature; on soft agar at the higher temperatures, inhibition of rate of growth became evident with increase of time; while in liquid media this slowing up of the rate of growth was delayed, the delay being greater with increase of volume. From considerable experimental evidence, Balls (3) concludes that this inhibitive effect of high temperature is occasioned entirely by the by-products from the metabolic process which accumulate at these temperatures, both within and without the cell, to such an extent that growth is limited by them, and he further suggests that the accumulation of such substances may play an important part in limiting the pathogenic power of the fungus at these higher temperatures. It is interesting to note that a similar decreased rate of growth with increase of time was found by Lehenbauer (9) to be an important factor in the growth of the corn plant at the temperature of 32° C. and above. The observations of this writer, however, were not continued over a sufficient period to give definite data on this point.

From a study of the data in Table VIII, it is clear that the temperatures shown to be most favorable for the growth of the fungus may be considered as optimum for a period of 96 hours only. Furthermore, as has been pointed out by Blackman (5), Lehenbauer (9), Leitch (10), and others, the optimum temperature value for growth under any specific set of conditions is quite without meaning unless the time limits for the period of the observations are accurately stated. Likewise, we are forced to conclude from these studies and from the work of Balls (3) that the actual optimum temperature for the growth of any strain of *Corticium vagum* will depend upon a large number of factors relating to the condition and history of the fungus, as well as to its specific environment.

TABLE IX.—*Ratio of increased temperature to rate of growth of the fungus Corticium vagum over a period of 96 hours*

Temperature range.	Comparative increase growth for each 10° C.	Coefficient.
°C.	Cm.	
4.6 to 14.6	0.76 to 6.5	8.50
8.2 to 18.1	1.35 to 8.1	6.00
10.9 to 20.1	2.87 to 12.1	4.20
14.6 to 23.1	6.50 to 15.0	2.30
16.3 to 25.3	7.80 to 17.5	2.24
18.1 to 29.8	8.10 to 12.5	1.54
19.8 to 29.8	10.25 to 12.5	1.22
20.1 to 31.2	12.10 to 10.0	0.82
23.6 to 32.6	15.00 to 6.5	0.43
25.3 to 35.0	17.50 to 0	0

The ratio at which the fungus growth increased with rise in the temperature is an especially interesting phase of the studies. Balls (3) found that the growth rate of the specific strain of the Rhizoctonia stage of *Corticium vagum* with which he worked accorded for a very short period with Van't Hoff's law. This same growth relation has been found true within certain limits for the pea by Leitch (10) and for the corn by Lehenbauer (9). In these studies a constant coefficient for each increment of 10° C. rise in temperature was not found; on the other hand, as shown in Table IX, a progressive decreasing coefficient resulted with increase in temperature. Owing in part to the operation of the possible "time factor" during the 96 hours of exposure, the specific value which might be predicted from the Van't Hoff law was approximated only within the narrow range between 14° and 25° C. What relation the progressively decreasing coefficient might have to the pathogenicity of the fungus is not clear, although it would appear significant in view of Balls's conclusions that "some (deleterious) products are formed at the low as at the high temperatures but at a much slower rate." Were such varying quantities of by-products present in the mycelium at the different temperatures as suggested, it would appear that they might be important in determining the varying degrees of closeness to which the hyphae of *Corticium vagum* grew to the substratum throughout the temperature range for fungal growth, a relation described in the following paragraph. As closeness of contact is of undoubted importance in the pathogenicity of the fungus, the decreasing ratio value might also have special significance as a factor in its pathogenic relation and be definitely indicative of greater parasitic possibilities on the part of the fungus at the lower temperatures where, owing to slow accumulation or absence of poisonous products, the hyphae adhere, and even embed themselves, in the substratum.

In addition to the fact that the hyphae grew exclusively from an apical zone, Balls (3) determined that the mycelia exhibited different growth characteristics at various temperatures. In liquid media at 20° C. he found that the hyphae grew straight and became slightly branched. At 34° in liquid media a fluffy mycelial growth resulted; more numerous short hyphae and fewer resting cells developed than at 20°. A number of these features were especially evident in the present study. At the lower temperatures the hyphae became sparsely branched and grew widely separated in the colony. The most characteristic feature lay in the fact that the hyphae at temperatures below 15° grew closely in contact with the medium (agar), and at still lower temperatures, as stated, definitely embedded themselves in it. Mycelia so developed appeared hyalin in color, and for considerable periods would remain almost indistinguishable from the media. Normal pigmentation and resting-cell formation were found to be greatly delayed at these lower temperatures as compared with the higher temperatures. With higher temperatures the mycelia branched more profusely and produced a more superficial type of growth upon the substratum. Between 24° and 28° profuse mycelial growth resulted frequently with definite aerial hyphae, often growing in a direction at right angles to the substratum. The characteristic brown pigment, together with the resting cells and consequent sclerotia formation, appeared at its maximum within this particular temperature range. These latter characteristics were found to decrease with increase of temperature above 27° and 28°, resulting in smaller colonies consisting of fluffy, aerial, short, but frequently branched, hyphae.

GENERAL CONSIDERATIONS

The uniform parasitic behavior of *Corticium vagum* on its several hosts in relation to the temperature of the soil is of special interest. Figure 6 shows the temperature range for the pathogenicity of *Corticium vagum* on the three hosts —pea, bean, and potato—in comparison with the temperature requirements for the growth of these plants.[12] It is evident that the optimum temperature for the growth of the bean, as undoubtedly as for the cotton (see Table I and fig. 1), is entirely outside the temperature range for dangerous pathogenic action of the fungus, and at such temperatures as would permit these plants to grow at their best, practically uninjured. On the other hand, the optimum for the continued growth of the pea and for the potato (18° C.) lies well within the range and approximates closely the temperature found most favorable for the pathogenic activity of the fungus. It would appear evident from the data accumulated, that this close approximation in the case of the pea and the potato is merely coincident, both the hosts in question being cool-temperature plants with similar temperature requirements. The fact that its optimum temperature for pathogenicity remains the same for the cotton and bean as for the pea and the potato would indicate that the temperature requirements for the parasitic relations of *Corticium vagum* and its various hosts is not influenced seriously by the species of hosts attacked, nor by the temperature requirements of any host, but is without doubt a condition determined primarily by a fixed physiological characteristic of the pathogen. It is further suggested that in general the resistance of these hosts as affected by the temperature of the soil plays a minor part in influencing the pathogenic state.

It is significant that the temperature range so far as determined for the parasitic activities of *Corticium vagum* agrees essentially with that found for its growth as a saprophyte in pure culture. While slight growth in pure culture occurs as low as 4.6° C., the severe damage which was found on the potato stems at 9° (*13*), the lowest temperature maintained, indicates that the fungus may become pathogenic under specially favorable conditions at temperatures considerably lower than 9° and probably as low as the minimum found for hyphal growth. Further, it should be recalled, in view of the relative low maximum (32.6°) for the growth of the fungus in pure culture, that under severe conditions, such as obtained for the pea and the bean in steam-sterilized soil, the highest temperature (29.5°) tried in the experiment did not entirely inhibit tissue destruction. It is important to note in this relation that a considerable difference appears between the optimum temperatures for pathogenic action and for hyphal growth (fig. 6). As previously indicated, however, we have no accurate data at present as to the possible optimum for growth of the fungus under the different conditions in the soil. It appears definite that the exact optimum for the saprophytic growth will vary within a considerable range, dependent upon a number of factors. Results obtained by Balls, together with the decreasing rate of growth noted at temperatures above 22.4°, suggest a possible optimum for saprophytic activity in the soil not far different from the low optimum found for its parasitic activity.

There appears no explanation of the fact, however, that the optimum temperature for tissue destruction on the various hosts falls considerably below that found most favorable for mycelial growth in pure culture.

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[12] Temperature data for the potato in figure 6 are obtained from the earlier publication of this series (*13*). The reader is referred to this article for a full discussion of this relation.

Balls points out that the sensitiveness of *Corticium vagum* to its own metabolic products would explain the decreased pathogenicity at the higher temperatures. Again, a decrease in pathogenic power may result from the definite tendency of the fungus to grow more superficially on the substratum at the higher temperatures, while closely adhering to the substratum at the lower temperatures. With respect to the optimum temperature for tissue destruction, it is quite probable that in the last analysis we are dealing with an enzym, or a group of enzyms, which are secreted more abundantly at 18° C., or which react more vigorously on the host tissue at this particular temperature. At present we have no direct evidence of such enzym activity of *Corticium vagum*. It is interesting in this connection, however, to note that Jones (9) obtained a more abundant secretion of cytolytic enzyms from *Bacillus carotovorus* at 18° to 21° than at the higher temperatures of from 25° to 28°. Studies of the enzymatic activities of *Corticium vagum* would undoubtedly throw much light on this particular relation.

The nature of the parasitism exhibited by *Corticium vagum* offers interesting material for speculation. In the first place, it is evident that in the injury and destruction of growing points of the potatoes and plumules of the pea and the bean, we are dealing largely with a question of escape. As has been suggested, the early growth of the various hosts in the soil above 21° C. is so rapid as to permit the tender bud portion to push through the soil with little or no injury. This was found especially true of the potato shoots. On the other hand, the lower temperatures retard the host growth and increase the exposure at just those temperatures which are most favorable for tissue destruction. Such retardation as shown is frequently disastrous. With tissue destruction in general, however, it appears that we are concerned with a specific type of parasitism determined, as stated, by a fixed character of the fungus and one closely allied to its saprophytic activity. What this fixed physiological factor, or factors, in the fungus is remains undetermined. Favorable soil temperature and closeness of contact, however, appear as important relations in this peculiar parasitism.

It seems reasonable to suggest that, given an opportunity for closeness of contact, the action of the fungus in the soil upon living tissue is not widely different from that on nonliving organic matter. Were such the case, it appears possible that in the pathogenic activities of *Corticium vagum* we are dealing with the action of one or a group of enzyms whose temperature relations differ but little, regardless of whether the substances acted upon under natural conditions of the soil belong to the living or nonliving organic world. The approximate coincidence of the temperature range for saprophytic growth and for parasitism, the apparent complete lack of host specialization, the similar temperature range for destruction of tissue of the various hosts, together with the peculiar nature of the lesions produced by the fungus, and finally the wide range of hosts, limited in number perhaps only by our lack of observation—all seem to support such a view. With *Corticium vagum* it appears that we are concerned with a peculiar type of parasitism quite distinct from that exhibited by the more specialized parasites. The recent works of Ames (*1*), Edson and Shapovalov (*7*), and Shapovalov (*16*), are interesting in the suggestion that a large number of saprophytes may destroy living tissues under certain conditions especially favorable for their growth.

SUMMARY

(1) Studies on the pathogenicity of *Corticium vagum* show that the fungus may become a vigorous parasite on the underground parts of both the pea and bean. The severity of damage resulting from the attack of this fungus on the two plants is shown definitely to be conditioned by the temperature of the soil.

(2) *Corticium vagum* may produce lesions on the pea through a soil temperature range of from 9° to 29° C. The greatest damage is found to result between 12° and 26°, with a definite optimum for tissue destruction at 18°. Essentially these same temperature relations are found for the pathogenic action of the fungus on the bean. Within the limits tried (9° to 29.5°) neither the high nor the low temperatures entirely inhibited injury from the fungus on bean stems, although, in unsterilized soil, damage to the bean was found to be limited to temperatures of 12.5°, 15°, 18°, and 21°, with a maximum amount of injury at 15° and 18°. Plumule destruction on the two plants, except under exaggerated conditions, occurred only at temperatures below 21°. These temperature values are practically the same as those found for the pathogenic action of *Corticium vagum* on the potato.

(3) Sterilized soil inoculated with a pure culture of *Corticium vagum* increased greatly the amount of injury caused by the fungus on both the pea and the bean. This is at variance with the results obtained in the studies of the potato.

(4) The cardinal temperature for the pathogenicity of *Corticium vagum* remained the same for the various hosts—potato, pea, bean, and cotton. Lesions occurred on all these plants from 9° to 29° C., with a general optimum for tissue destruction near 18°. The growing-point and plumule destruction on the various plants was exhibited at practically the same range of soil temperatures for all hosts studied. These various temperature relations held true for all the different strains of the fungus used in the experiment.

(5) The temperature requirements for the pathogenic action of *Corticium vagum* on its various hosts appear definitely as a fixed inheritable characteristic of the fungus, more or less independent of the temperature relations of the host on which it becomes parasitic.

(6) *Corticium vagum* grows in pure culture from 4.6° to 32.6° C. with an optimum for a period of 96 hours between 25° and 27°. The growth rate of the mycelium on hard agar between 23.6° and 32.6° decreases with the time of exposure. This fact suggests a much lower optimum for the continued growth of the fungus. At the lower temperatures hyphae embed themselves in the artificial substratum and retain their hyalin color and active state much longer than at higher temperatures. At 20° to 30° the mycelial growth is much more superficial, producing aerial hyphae which grow frequently at right angles to the surface of the medium. This closeness of growth in contact with the substratum at the lower temperatures probably plays an important part in the ability of the fungus to attack living tissues at temperatures below 21°.

(7) The temperature range indicated by the minimum and maximum temperatures for the pathogenicity of *Corticium vagum* approximates closely the temperature range found for its saprophytic activities. The optima for these physiological processes, however, vary widely. The temperature optimum for mycelial growth of the fungus, in fact, appears

to bear no direct relation to the temperature requirements for maximum pathogenicity. The ratio of the rate of growth with increase of temperature conforms to Van't Hoff's law only between the temperatures of 14° to 24° C.
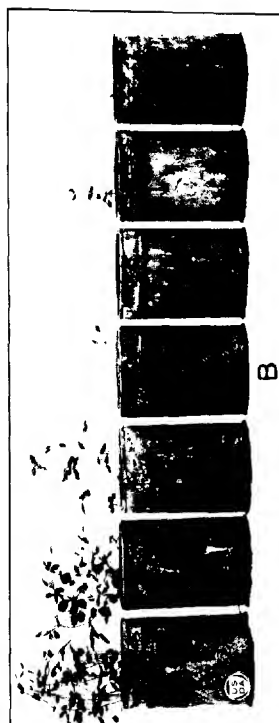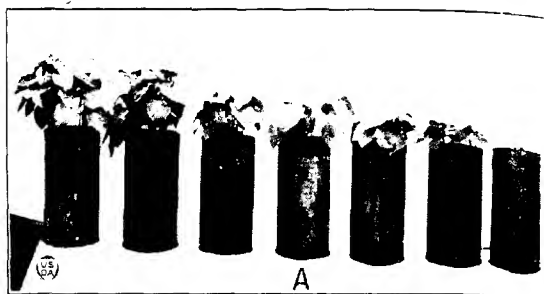
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PLATE I

PLATE 2

A.—Bean plants from Experiment 2 grown in uninoculated soil at temperatures of 29.3°, 26.6°, 23.5°, 21.5°, 18.5°, 16°, and 13.2° C. Germination and growth occurred throughout the entire range. Fifteen seeds were grown in each pot. Dry-weight determination and height of plants are recorded in Table VI.

B.—Beans grown in soil inoculated with *Corticium vagum* and held at the various temperatures as indicated in A. Very slight injury occurred at 29.3° C. (See Table VI.)

C.—Cotton plants which survived in infected soil at the various temperatures of 28°, 26°, 23.5° 18°, and 15° C. Seven seeds were planted in each pot. At the same temperatures in soil to which *Corticium vagum* had not been added the control plants grew perfectly, except that at 15° C. only half of the seeds germinated. (Photograph provided by F. R. Jones.)

GROWING EXPERIMENTAL CHICKENS IN CONFINEMENT[1]

By C. A. HERRICK, *Assistant in Parasitology*, J. E. ACKERT, *Professor of Zoology and Parasitologist*, and BERTHA L. DANHEIM, *Assistant in Zoology, Kansas Agricultural Experiment Station*

INTRODUCTION

This paper is an outgrowth of a research begun in 1916 by one of the authors (Ackert), who undertook a series of studies on the life histories of fowl cestodes. Early in the work it was realized that the raising of large numbers of chicks in confinement presented a problem in itself. Although the chicks made rapid gains during the first two months, they soon began to show signs of lameness, and in a few weeks became so weakened and abnormal as to jeopardize the experiments. On consulting the literature of the subject, it was found that other investigators had experienced somewhat similar difficulties.

Drummond[2], after a series of tests, concluded that a diet sufficient to produce normal development under range conditions was insufficient for chicks raised in the laboratory. By using a mixture of chicken feed, cabbage, and charcoal, and by replacing the water with milk, Funk[3] was able to reduce greatly the mortality, even though the chicks were kept in small cages. Osborne and Mendel[4] succeeded in raising to maturity some apparently normal fowls by supplying crude fiber in the form of paper pulp and blotting paper as a "ballast." Difficulties most nearly identical with those of the present writers were experienced in the rearing of chicks by Hart, Halpin, and Steenbock,[5] who state that "In the rearing of baby chicks in confinement a difficulty serious and obscure in its etiology is the one characterized by poultrymen as 'leg weakness.' This trouble usually develops in 4 to 6 weeks after hatching, but the writers have seen it show itself 4 to 6 weeks later. The principal symptoms shown by the bird are, first, an unsteady gait, developing into difficulty of locomotion with a tendency to remain squatted a good part of the time; a pronounced ruffled condition of the feathers; an anemic condition of the wattles and comb; and a swelling of the leg joints, which is sometimes permanent. A loss of appetite accompanies these conditions and usually death follows suddenly." These symptoms are very similar to those shown by the earlier lots of the writers' chicks, except that they retained a good appetite for grain and often lived for several weeks.

Hart, Halpin, and Steenbock, by incorporating 10 per cent of paper in the ration, succeeded in raising normal chicks in confinement for 18

Journal of Agricultural Research,
Washington, D. C.
agj

Vol. XXV, No. 11
Sept. 15, 1923
Key No. Kans.—87

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weeks, at the end of which time they were discarded. The method was so successful for those investigators that the present writers attempted to use it, but, owing to some unknown cause, the chicks discriminated against the feed containing the paper and failed to eat an amount sufficient to prevent leg weakness. After many trials, the method about to be described of raising chicks in confinement was developed, and, since it has been successful with rather large numbers of fowls, it is described with the hope that it may be of assistance to other experimenters. The writers wish to express their appreciation of the friendly cooperation of Dr. J. S. Hughes and Prof. L. F. Payne.

EQUIPMENT

The parasitology animal house in which the chicks were raised occupies a portion of the first floor of a two-story frame building. Screens on windows and door aid in excluding flies and other insects from the house. In the interior a hallway separates the sterilizing room, feed room, and laboratory on the north from the four rectangular pens on the south. Each pen, separated from its fellow by a screened partition, is 8 feet wide by 32 feet long, and extends north and south. Ample light and ventilation are afforded by large windows on all sides except the west. The house is steam-heated and is usually kept just warm enough to prevent freezing during the coldest weather. For small chicks, both kerosene and electrically heated hovers have been utilized, but at present a high-pressure steam radiator with a conical tin cover is placed in the partition between each pair of pens (Pl. 2, B, right).[6] Detachable canvas curtains aid in retaining the warm air in these hovers. For the older chickens, the roost and dropping board are placed along one side of the pen so that the fowls may select places out of the drafts. In the summer the screened partitions tend to increase the heat, but by spraying the pens with cold water the temperature is so reduced that the chicks suffer little from the heat.

CHICKENS

In the early experiments three breeds of chickens were used: Barred Plymouth Rocks, Buff Orpingtons, and Single Comb White Leghorns. The weight and comparative inactivity of the first two breeds seemed to make them more susceptible to leg weakness, for they showed abnormal symptoms sooner than did the alert Leghorns, though all were kept in the same quarters. After the first two years, therefore, only Single Comb White Leghorns were used.

All of the chickens were hatched in incubators, and placed at once in the pens. Lots of baby chicks have been secured from local poultrymen, the College Poultry Farm, and from commercial hatcheries.

As is well known, excellent care of baby chicks during the first two weeks is imperative. A temperature of about 100° F. is maintained in the hover. Sand, oyster shell, and clean water are kept constantly before them and a small amount of feed is given about every two hours. Shallow drinking fountains which exclude the chicks have the advantages of freedom from contamination and of keeping the chickens dry. As the chicks grow older, the board floor, 2 feet above the ground, is gradually covered with a litter of wheat straw. The fine straw and droppings are removed

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[6] Photographs by F. E. Colburn.

each week and fresh straw added, so that the scattered grain disappears in the litter, thus making the chickens scratch for the feed. When the chicks attain the age of two to three weeks, dry mash is kept before them in a hopper, and containers of sand and oyster shell are also provided. Green feed and skim milk are given at least twice a week.

HISTORY OF THE LOTS OF CHICKS

During 1916–1918 the experimental chicks were grown in a small isolated house (Ackert)[7] with cement walls and floor, the latter being covered with sand. The baby chicks were started in this house only during the spring and summer months. When fed on rations of cereals, bone ash, and green alfalfa they thrived during the first eight or ten weeks; then lameness and other disorders began to develop. Since many of the parasitized chicks or their controls died in these crowded quarters before the termination of the experiments, the present animal house was provided.

The next year (1919) the experiments were conducted in the parasitology animal house already described. In these light, roomy quarters, with rations adapted from Lippincott,[8] good results were obtained. During the first week the baby chicks were given soft feed, such as bread or corn pone mixed with rolled oats and moistened with skim milk. The amount of rolled oats was gradually increased and such grains as wheat and cracked corn were added until the chicks were about two and a half or three months old, when they were put on a regular ration of hopper-fed dry mash, scratch grain in the litter, green alfalfa and skim milk. The dry mash consisted of five parts of shorts, five of bran, three of oil meal, and one of bone ash. The scratch grain was a mixture of two parts of cracked corn, two of wheat, two of kafir, and one of oats. Liberal amounts of fresh, green alfalfa, cut into half-inch lengths, were fed daily, and skim milk was before them practically all of the time. In the winter sprouted oats supplanted the green alfalfa.

In this lot of chicks only 3 died of leg weakness. The remaining ones (29 pullets and 2 cockerels) developed normally, averaging in weight 1,383.5 gm. at 1 year of age. One pullet began to lay when 5 months and 8 days old; and in 8 months, from November 14 until July 16, the lot laid 195 dozen eggs, although the egg production of 17 of the pullets was considerably reduced because from March 28 to May 28 they received no alfalfa and no skim milk. One of these hens is shown in Plate 2, A.

Fifteen chickens from this lot were kept in these pens for three years, and although not fed for heavy egg production, they laid well until they were discarded. At that time the ovaries of all but two contained well-developed eggs. One of the two had several partly reabsorbed eggs in its body cavity, while the other, which had a small ovary, had probably never laid many eggs. The largest hen (Pl. 1, B, left) weighed 3,288.6 gm., and the smallest (Pl. 1, B, right, front) 1,360.8 gm. The lot, a few of which are shown in this figure, averaged in weight 2,041.4 gm. The cock (Pl. 1, B, center), a vigorous bird, weighed 2,721.6 gm. The fertility of the eggs from these hens was high. A lot of 25 eggs incubated at the College Poultry Farm in February, 1920, were

[1] ACKERT, James E. ON THE LIFE CYCLE OF THE FOWL CESTODE, DAVAINEA CESTICILLUS (MOLIN). *In* Jour. Par., v. 5, p. 41–43, pl. 5. 1918.
[8] LIPPINCOTT, William Adams. POULTRY PRODUCTION. Ed. 2, rev. 517 p., 233 fig., 1 map. Philadelphia and New York. 1916.

all fertile, and yielded a hatch of 18 strong chicks. They were of average normal size, exceeding in weight an equal number of chicks taken at random from the College Poultry Farm. Their gains in weight likewise slightly exceeded those of the latter. These chicks (offspring of those grown in confinement) grew to maturity, laid at the usual age, and behaved normally in all respects. One of them is shown in Plate 1, A.

More rapid growth and generally better results were obtained in 1921 by beginning with buttermilk chick feed mixed with skim milk, and gradually changing to a ration of equal parts of wheat and corn chop as a scratch grain and a hopper-fed dry mash consisting of equal parts of corn meal, bran, shorts, beef scraps, and one-third part bone meal. This lot received green alfalfa after the first week and skim milk after the second. Although these chicks were in transit eight days, having their vitality greatly reduced, they averaged in weight 163.3 gm. in seven weeks and attained an average weight of 1,927.9 gm. at the age of 7 months. They laid their first eggs when 5 months and 22 days old, and continued to lay well until they were discarded in May, 1922. Plate 2, B, shows a group of these fowls shortly before they were discarded. Similarly, in May, 1922, 150 baby chicks shipped from Michigan were started on the buttermilk chick feed. From the first these chicks made gains which compared favorably with the average daily growth for chickens of this breed. Under conditions similar to those of the previous lots, 125 of these fowls reached maturity without any sign of leg weakness, and began to lay at the early age of 4 months and 27 days.

Some workers have experienced greater difficulties from leg weakness and other disorders with chicks hatched in late summer or fall than with those hatched earlier in the year. This has not always been true here. In the fall of 1921, 50 baby chicks were secured from Ohio. They received the same care as those hatched in the spring, and, although some were smothered, none of the survivors showed the slightest signs of leg weakness. They began to lay when 5 months and 7 days old, and the ovaries of all but one contained well-developed eggs when the chickens were discarded the following May.

However, in September, 1920, a lot of 50 chicks made good gains for about eight weeks, then they began to suffer from leg weakness. Soon all contracted it, and many died or were killed. Nine that recovered began laying at the age of 5 months and 4 days. Concerning the onset of the attack, the first abnormal symptoms appeared when the chicks were rapidly increasing their weight and plumage. Restlessness and perverted appetites appeared, the chicks showing an avidity for grain and an aversion for green feed and skim milk. The lameness and other characteristic symptoms soon followed. Whether or not the season of the year (fall) was effective in producing the abnormal appetite of these chicks is problematical, but the short, more or less cloudy days of late autumn are not conducive to activity for fowls raised in confinement.

DISCUSSION

Among the apparent factors of the production of abnormal chickens in the first experiments here were insufficient litter and crowding. In the course of two months these factors probably reduced the activity of the chicks, and paved the way for perverted appetites and leg weakness. With these difficulties obviated, and under the conditions described, several hundred chickens have been raised to maturity without develop-

ing leg weakness or any other apparent disorder. That they were normal seems evident from their thrifty appearance—their smooth feathers, clear eyes, and good color of comb and wattles. An average lot is shown in Plate 2, B. Their activities of scratching, dusting, brooding, crowing, and fighting, and their age of maturing, their weight, egg production, and fertility, all indicate that they were normal Single Comb White Leghorns. Moreover, 15 fowls from one lot thrived under these conditions for three years, when they were discarded.

On the other hand, the idea must not be entertained that here is an easy method of raising experimental chickens and that leg weakness has been banished. Unless there is ample light, ventilation, room and cleanliness, and unless the rations and care are administered consistently, especially during the first three months, discontent, inactivity, diminished or perverted appetites and leg weakness are almost certain to appear.

SUMMARY

(1) While experimenting in 1916 with chickens raised in confinement, a disorder known to poultrymen as leg weakness developed in the flock and interfered with the experiments.

(2) Single Comb White Leghorns proved to be less susceptible to the ailment than the heavier breeds, and were used for all subsequent experiments.

(3) Chicks hatched in incubators were placed at once in screened pens. Best results have come from the use of guaranteed chicks from commercial hatcheries.

(4) Marked improvement in the results of raising the chicks was accomplished in 1919 by the aid of well-lighted, screened pens, in a steam-heated frame building, and by the adoption of an adequate diet, consisting of common grains, a dry mash, green feed, skim milk, oyster shell, charcoal, and water.

(5) Light, roomy, well-ventilated pens with clean litter (preferably wheat straw) are valuable assests in keeping the chickens active and healthy.

(6) Under such conditions several hundred experimental chickens have been raised to maturity without showing any abnormal symptoms. Their growth, behavior, egg-production and weight have been similar to such performances of average Single Comb White Leghorns. From one lot, 15 individuals thrived in the pens three years, their eggs yielding normal hatches and their chicks developing regularly.
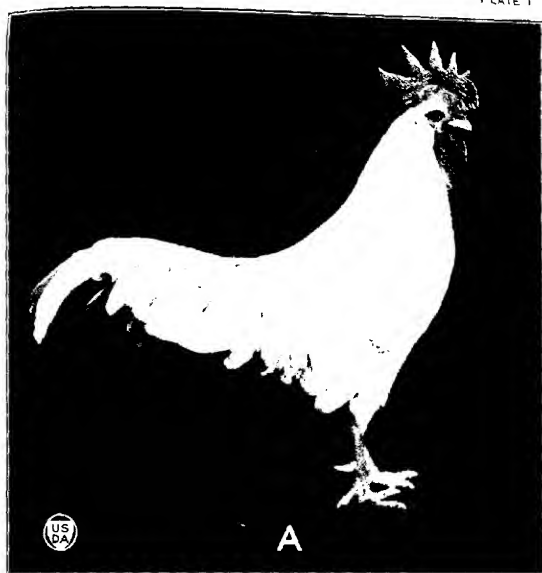
(7) The contention that chicks hatched in late summer or fall do not develop as well as spring chicks is partially supported, as leg weakness since 1919 has been confined to a lot of fall chicks. Reduced activity, possibly due to short, cloudy days, seems to lead to diminished or perverted appetites. On the other hand, no leg weakness or other disorder developed in a lot of chicks hatched in the fall of 1921.

(8) Failure to take liberal quantities of green feed and skim milk, either from lack of supply or loss of appetite, seems to induce leg weakness in two to three months' old chicks that are rapidly increasing their weight and plumage.
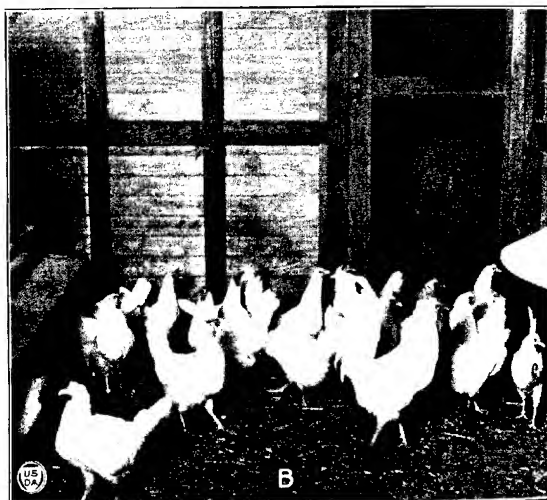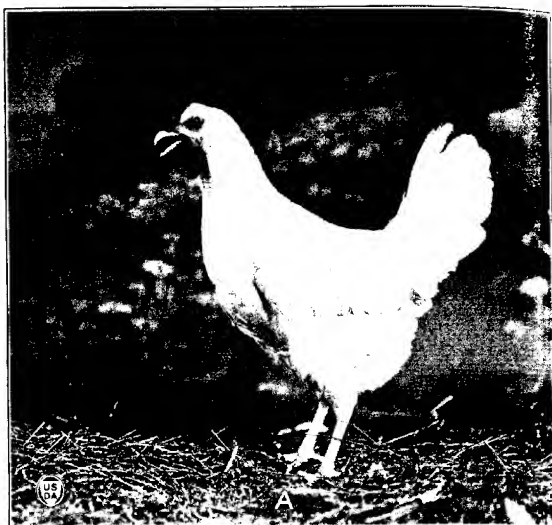
A

B

A

B

PLATE 2

A.—Hen during second year of confinement.
B.—Seven and eleven months' old chickens grown in confinement.

ACIDITY OF CORN AND ITS RELATION TO VEGETATIVE VIGOR [1]

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INTRODUCTION

Some investigators are inclined to believe that there is but little variation in the cell-sap reaction of healthy plants of a given species, and that the hydrogen-ion concentration of each cell remains practically the same during its life (2).[2] The well-known facts that blood is so buffered that in a healthy individual its reaction never varies more than a few hundredths of a P_H unit from the norm, and that acidosis leads to profound physiological disturbances lend color to the supposition that the same might be true of plant juices. Consistent with this is the statement of Truog and Meacham (16), that for each species of plant there undoubtedly is a certain acidity which is most favorable for the life processes of that particular species. That this optimal reaction of the plant is susceptible to change is suggested by Clevenger (5) who says, "It seems possible that certain conditions may change the acidity of the plant juice sufficiently to produce an acidity which is unfavorable for the plant." Moreover, Haas (8) is led by his work on sweet clover, which showed a difference in vigor between the first and the second year's growth, and a higher hydrogen-ion concentration in the less vigorous plants, to ask the question, "Do the more vigorously growing plants of a species show a decrease in the actual reaction of their juice compared with that of less actively growing plants of the same species?"

This same question was very strongly suggested by the first few experiments of the present investigation, originally designed to determine whether or not there are varietal differences in the acidity of corn (maize) seedlings. A surprising variability in the acidity values [3] of plants of the same variety precluded conclusions on the latter question, but focused attention on an apparent correlation between the measurements and vegetative vigor.

PROCEDURE AND METHODS

Ten strains of Reid Yellow Dent corn (*Zea mays*), self-pollinated one year, were selected for these experiments by Dr. G. N. Hoffer at La Fayette, Ind. Dr. Hoffer, referring to the growth of these strains in Indiana, describes the plants of No. 31 and 972 as vigorous, without

Journal of Agricultural Research,
Washington, D. C.
wjk

Vol. XXV, No. 11
Sept. 15, 1923
Key No. G-328

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any apparent disease symptoms; No. 113, 129, and 326 as stunted, with spotted or streaked, or prematurely dead leaves; No. 150, 350, and 507 with various abnormalities of leaf and stalk, indicating susceptibility to disease; and No. 315 and 485 with a marked tendency to fall down. Four successive plantings of each of the 10 strains were made in greenhouses and a fifth in an outdoor plot, on the grounds of the Department of Agriculture at Washington, D. C. Growth conditions in these plots were not equally favorable for corn, hence the five series were strikingly different in vigor.

The plants, grown to the age indicated in the different experiments, were cut about 2 inches from the ground, and after being carefully freed from dust and dirt, were ground in a food chopper. The juice was squeezed from the pulp by hand through clean muslin and its acidity determined immediately. In the earlier experiments, the juice from the whole top was expressed as one sample, but later, when the quantity of material permitted, the juice from the leaves and stalks was expressed separately.

All hydrogen-ion determinations were made electrometrically. The major details of the method have been given in an earlier paper (*11*). The titrations of total acidity also were made electrometrically, a uniform procedure being followed in all cases. One-twentieth normal sodium-hydroxid solution was added, 1 cc. at a time, to a 10-cc. sample of undiluted juice by means of a burette, the tip of which was inserted through the cork in the electrode vessel. The P_H values corresponding to the potential differences resulting from each successive addition of alkali to the sample were plotted against the corresponding quantities of sodium hydroxid. The quantity required to bring the hydrogen-ion concentration to P_H 8.3, the turning point of phenolphthalein, was taken to represent the titratable acidity.

In order to discover whether a relation existed between the acidity of the juice and its density, specific-gravity determinations were made for each sample in the later experiments. For these determinations the juice was filtered and refiltered through a folded filter until clear, precautions being taken to prevent excessive evaporation. The density was determined by means of a small pycnometer in the form of a glass capsule with capillary intake and outlet tubes. The density is referred to water at the same temperature as that of the juice.

RESULTS

SERIES I

The first set of plants was grown in medium to fine sandy loam [4] with a neutral reaction (P_H 7.0), in a greenhouse bench on which the soil was approximately 10 inches deep. By the sixth week, when the plants were cut, they had reached an average height of 50 cm. On the whole, the plants were normal and in good condition. Strains No. 113, 31, and 326, however, were less vigorous than the rest. Either two or three plants, depending on their size, were cut for each sample. One set of determinations was made in the morning, and in order to compensate for any error from differences in the time of cutting and order of expres-

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[4] I am indebted to Mr. L. A. Hurst, of the Office of Soil Fertility Investigations, Bureau of Plant Industry, for the technical descriptions of the soils used in this investigation.

sion, duplicate determinations, but with their sequence reversed, were made on the same strains each afternoon. The results are given in Table I.

TABLE I.—*Acidity of the tops of corn plants of Series I, 36 to 39 days old,[1] in good condition, grown in the greenhouse in neutral sandy loam*

Strain No.	Date of determination (May, 1922).	Morning cuttings.		Afternoon cuttings.	
		Titratable acidity (cc. $N/20$ NaOH).	P_H	Titratable acidity (cc. $N/20$ NaOH).	P_H
972	27	4. 5	5. 68	4. 4	5. 68
129	25	4. 9	5. 54	5. 0	5. 61
507	27	4. 8	5. 60	5. 5	5. 58
350	24	5. 0	5. 55	5. 0	5. 56
485	24	5. 0	5. 59	6. 0	5. 46
150	27	5. 6	5. 52	5. 7	5. 55
315	26	5. 8	5. 48	5. 7	5. 44
113	25	6. 1	5. 42	5. 6	5. 45
31	25	5. 8	5. 56	6. 6	5. 50
326	26	[2](7. 4)	[2](5. 38)	6. 6	5. 44
Average		5. 3	5. 55	5. 7	5. 53

[1] Throughout this paper age is reckoned from the date of planting.
[2] Poorest plants in the plot, described as "stunted, leaf tips dead," not included in the averages.

The close agreement in the values obtained from morning and afternoon cuttings brings out the interesting fact that neither the hydrogen-ion concentration nor the titratable acidity changed measurably between 8 a. m. and 12.30 p. m. In those cases where the measurements were not the same, as many show a decrease as show an increase. Evidently the diurnal change in acidity, reported in many plants, is not marked in corn grown under these conditions.

SERIES II

The second set of plants was grown in another greenhouse in a sandy clay loam with a slightly acid reaction (P_H 6.5), and only 3 or 4 inches deep on the bench. This series, like the preceding one, reached an average height of 50 cm. by the sixth week, at which time the plants were cut. However, the stalks were spindling and the leaves narrow, whereas the plants of the first set had stout stalks and the broad leaves of normal plants of that age. As the plants were cut, they were weighed for comparison with those of subsequent series and found to average 44 gm. Green weight expresses more accurately than does height the rate of growth and health of the plant (7).

Either one or two plants, depending on their size, were taken for each sample and the stalks and leaves expressed together. Unfortunately, there was not sufficient material to make duplicate measurements on strains 326 and 113, and the plants of No. 485 and 350 were lost. The acidity determinations for this group of plants are recorded in Table II.

TABLE II.—*Acidity of the tops of corn plants of Series II, 37 to 44 days old, in poor condition, grown in the greenhouse in slightly acid sandy clay loam* [1]

Strain No.	Date of determination (June, 1922).	Titratable acidity (cc. N/20 NaOH)	P_H	Averages.	
				Acidity (cc. N/20 NaOH).	P_H
972	9	5. 7	5. 40	6. 3	5. 34
	12	6. 9	5. 29		
	8	6. 4	5. 21		
507	14	(8. 5)	(5. 10)	6. 4	5. 25
	15	6. 4	5. 29		
	8	6. 5	5. 28		
31	13	6. 6	5. 25	6. 4	5. 25
	14	6. 2	5. 22		
	10	6. 5	5. 33		
129	12	6. 7	5. 23	6. 6	5. 28
	13	(9. 0)	(5. 10)		
326	9	6. 7	5. 30	6. 7	5. 30
	8	6. 4	5. 30		
315	9	5. 9	5. 38	6. 8	5. 33
	14	7. 3	5. 34		
	15	7. 5	5. 30		
	10	7. 0	5. 23		
150	12	7. 2	5. 19	7. 5	5. 19
	13	8. 8	5. 10		
	13	7. 0	5. 24		
113	8	7. 5	5. 15	7. 5	5. 15
Average		6. 8	5. 26		

[1] Measurements in parentheses are not included in the averages because they were made on exceptionally poor spindling plants, not typical of the row.

The outstanding feature of Table II is the high acidity of all the plants compared with the more vigorous ones of Table I. Additional evidence of an interrelationship of acidity and vigor of growth was afforded by the notes on relative development of some of the individual plants constituting the group. Some highly acid plants of 507 and 129, the measurements of which are inclosed in parentheses in the table and not averaged because the plants did not seem typical of the strains, were described, respectively, as "spindling stalk, narrow leaves, tips dying," and "short and spindling, poorest of all." Of the uniformly highly acid strains, No. 150 showed a tendency to become diseased in all the plantings, while No. 113 was nearly always at the bottom of the list in vigor.

SERIES III

To check the results given in Table II a third series had been planted between the rows of the second series before the plants were cut. The soil reaction was found to have become more acid, being P_H 6.2 as compared to P_H 6.5, the value obtained a month before. The plants grew even more poorly than those of Series II, having spindling stalks, and narrow leaves with dry, dead tips, the lowest dying early. The average height of the group did not reach 50 cm. until the seventh week when the plants were cut and found to have an average green weight of 44 gm., the same as that of the plants of Series II at six weeks. It was necessary, because of the small quantity of juice in each plant, to take from two to

ive plants for each sample. There were not enough plants of No. 113
or even one determination. The results are given in Table III.

TABLE III.—*Acidity of the tops of corn plants of Series III, 44 to 56 days old, in very poor*
condition, grown in the same soil as Series II [1]

Strain No.	Date of determination (July, 1921).	Titratable acidity (cc. N/20 NaOH).	P_H	Specific gravity.
37	8	6. 9	5. 23	1. 0246
	8	(9. 2)	(5. 22)	(1. 0253)
35	7	9. 4	5. 03	1. 0221
	18	(12. 3)	(5. 01)	(1. 0201)
30	7	9. 5	5. 16	1. 0232
50	6	10. 2	5. 10	(2)
19	14	10. 6	5. 13	1. 0248
15	11	10. 8	5. 06	1. 0194
1	6	11. 0	5. 10	(2)
	11	(13. 2)	(5. 01)	(1. 0272)
16	18	11. 7	5. 03	1. 0246
972	14	12. 3	4. 97	1. 0247
Average		10. 3	5. 09	1. 0233

[1] Figures in parentheses are from the poorest, most spindling plants, not typical of the row, determined separately and not included in the averages, but given to show the greater acidity of the less vigorous plants.
[2] Not enough juice for determinations.

Comparison of the results reported in Table III with those in Table II
shows that the plants have a markedly increased titratable acidity and
hydrogen-ion concentration, although grown in the same soil. But these
plants grew more slowly and were more spindling than their predecessors,
besides showing an abnormal dying of the tips of the leaves. In Table III,
as in the two preceding tables, are given in parentheses the values ob-
tained from the poorer plants of each group which obviously were below
the average in vigor. These data afford additional evidence of an inter-
relation of vigor and acid accumulation.

SERIES IV

The fourth series was a repetition of the first in the original greenhouse
to see whether, on second trial, the growth conditions there would again
produce the more vigorous, rapidly growing plants with their compara-
tively low acidities. Unused soil adjacent to that of the first plot was
used for Series IV. A redetermination of the soil reaction showed that
the soil on this bench remained approximately neutral, P_H 7.2.

The higher temperatures of June and July, as compared with those of
April and May, probably were responsible for the fact that the plants of
this series made an even more rapid growth than did those of Series I.
At the end of only five weeks their average height was 80 cm. and their
average green weight 126 gm. as compared with 50 cm. and 44 gm., the
corresponding measurements for the unhealthy Series III at the age of
seven weeks. The data are given in Table IV.

TABLE IV.—*Acidity of the tops of corn plants of Series IV, 32 to 35 days old, in good condition, grown in the greenhouse in sandy loam, P_H 7.2*

Strain No.	Date of determination (July, 1922).	Titratable acidity (cc. N/20 NaOH).	P_H	Specific gravity.
485 (a)[1]	26	3. 7	5. 51	1. 013
485 (b)[1]	28	4. 3	5. 49	1. 015
113	27	4. 3	5. 49	1. 013
507	25	4. 6	5. 44	1. 014
326	26	4. 7	5. 56	1. 015
315	26	4. 7	5. 49	1. 013
350	27	4. 8	5. 55	1. 014
31 (a)[1]	28	4. 8	5. 43	1. 015
31 (b)[1]	28	5. 6	5. 34	1. 016
129	25	5. 0	5. 51	1. 0145
972	27	5. 1	5. 51	1. 0163
150	25	5. 2	5. 38	1. 0152
Average		4. 7	5. 48	1. 0149

[1] Strains 485 and 31 were uneven in growth, so the best plants of each row (a) were determined separately, and when compared with the rest (b) show a greater acid content in the less vigorous plants.

Table IV shows that the juice of the plants of this series had a hydrogen-ion concentration practically the same as that of the vigorous Series I and a slightly lower total acidity, while both the hydrogen-ion concentration and the total acidity were very much lower than in the weak spindling plants of Series II and III.

As these large, rapidly growing plants seemed so much more succulent than the spindling, slow-growing ones of Series III, it was not surprising to find the specific gravity measurements of their juice very much lower than those of that series. This fact suggests that the low acidity of the former compared to the latter may be due in part to a greater water-absorbing capacity of the rapidly growing plants, resulting in greater hydration of the tissues and dilution of the cell sap. It is interesting to note in this connection that Reed (*13*) found the concentration of the sap of certain trees to vary inversely with the rate of growth.

SERIES V

The fifth planting was in an outdoor plot for comparison with the greenhouse plants. The soil was a fine, sandy loam, slightly acid, with a P value of 6.6. The season was too far advanced for corn, the cool night of September and October so inhibiting its growth that the plants were poor and more stunted than those of any other series. They were characterized by narrow leaves and by very short internodes. Many were leaning or fallen.

To facilitate comparisons of the acidity determinations of these slow growing plants with those of vigorous ones of the same age, the average of the acidity measurements for each strain are given in Table V alongside the corresponding values for plants of Series IV, which had been allowed to grow in the greenhouse until they were 8 weeks old.

TABLE V.—*Comparative acidities of the stalk and leaf juices of vigorous and of stunted corn plants, 8 weeks old*

[Series IV, vigorous, average height 150 cm., green weight 311 gm.]
[Series V, stunted, average height 60 cm., green weight 124 gm.]

Strain No.	Stalks.						Leaves.					
	Titratable acidity (cc. $N/20$ NaOH) of series—		P_H of series—		Specific gravity of series—		Titratable acidity (cc. $N/20$ NaOH) of series—		P_H of series—		Specific gravity of series—	
	IV	V	IV	V	IV	V	IV	V	IV	V	IV	V
93	1.5	4.8	5.68	5.22	1.0183	1.0172	9.8	9.3	5.45	5.46	1.0166	1.0225
48[1]	3.0	8.0	5.41	5.03	1.0193	1.0197	10.2	11.6	5.37	5.40	1.0238	1.0296
31	3.3	5.6	5.50	5.25	1.0166	1.0190	9.4	10.3	5.52	5.46	1.0216	1.0258
97	3.5	4.3	5.35	5.25	1.0180	1.0248	10.4	10.9	5.43	5.47	1.0239	1.0253
130	2.3	6.9	5.69	5.19	1.0176	1.0202	9.0	11.9	5.43	5.42	1.0313	1.0261
98	2.5	8.2	5.59	5.16	1.0134	....	8.4	14.9	5.52	5.33	1.0204	1.0261
35	3.6	6.0	5.35	5.21	1.0166	1.0174	10.4	9.8	5.31	5.43	1.0234	1.0229
119	2.8	6.6	5.44	5.16	1.0143	1.0144	10.2	10.7	5.37	5.44	1.0236	1.0197
113	1.8	......	5.60	......	1.0194	....	9.6	......	5.40	......	1.0211	......
Average	2.8	6.3	5.51	5.19	1.0171	1.0175	9.7	11.1	5.42	5.43	1.0229	1.0248

[1] The plants of strain 48s in Series V appeared wilted when cut. This probably accounts for the unusually high acidity and hydrogen-ion concentration of the stalks and the high specific gravity of both stalk and leaf juice of those plants.

The data in Table V show that the stalk juices of the rapidly growing greenhouse plants of Series IV had a decidedly lower titratable acidity and hydrogen-ion concentration than did those of the stunted ones from the outdoor plot (Series V). That the stalks of these slow-growing plants are characterized by a relatively high acid accumulation rather than simply a high sap density due to an increased concentration of all the solutes is shown by the fact that the specific gravity figures bear no consistent relation to the acidity measurements.

In some of the strains there is no significant difference in the acidity values for the leaf juices of the good and the poor series. The acidity of the stalk appears to be affected in greater degree by the environment than does that of the leaves. There are some data reported in the literature pointing to this same fact. Thus, with different fertilizer treatments, Bauer and Haas (*3*) obtained variations in the actual acidity in the stalks of corn between P_H 5.31 and 5.95; and in the leaves between 5.31 and 5.49. Haas (*8*) found also that liming the soil decreased the actual and total acidities of the juice of the stems and petioles of red clover plants more than it did that of the leaves.

Consistent with the evidence that the concentration of titratable acid is higher in the leaves than in the stalk of the same plant are the specific-gravity measurements in Table V, which show that in every plant of each series, whether vigorous or stunted, there is a much higher sap density in the leaves than in the stalk. However, in all the stunted plants of Series V the hydrogen-ion concentration of the stalk juice was much higher than that of the leaves, although the titratable acidity was very much lower. In the vigorous plants of Series IV the lower concentration of hydrogen-ions accompanied the lower titratable acidity in the stalk, except in a few instances where the P_H values were practically the same. Furthermore, the ratio of the titratable acidity of the stalk to that of the leaves, which was 1 to 3 or 1 to 4 and sometimes even

smaller in the rapidly-growing plants of Series IV, was 1 to 2 or greater in the slow-growing plants of Series V.

To illustrate these differing characteristics of the plants of Series IV and V and also the extreme variations which are found in the acidity of plants of the same strain, some titration curves obtained with plants of No. 326 are given in figure 1. The data for stalk and leaves of a plant of less than average acidity from Series IV are plotted alongside those of a plant of more than average acidity from Series V.

The slope of the curve representing the stalks of the vigorous plants is steeper than that of the slow-growing ones and shows that the titratable acidity of the latter is more than three times that of the former. The curves representing the leaf acidities show a similar relation. The curve for the stalk juice of the poor plant starts below but crosses that of the
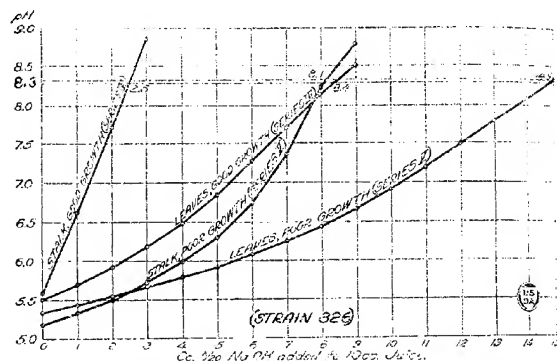

FIG. 1.—Electrometric titration curves of leaf and stalk juices of Reid Yellow Dent corn from vigorous plants (Series IV) from the greenhouse and stunted plants (Series V) from the field.

leaves, a relation shown by the curves for the leaves and stalk of every plant in Series V, but for none of the plants of the vigorous series.

The acidity data for strain No. 150 are not included in Table V because, in both Series IV and V, these plants appeared diseased and not comparable with the others. The leaves were crinkled, spotted, and dead at the tips, with the lower leaves prematurely dead. In Series IV, both the hydrogen-ion and titratable-acid concentrations in these leaves were definitely higher than in any others, being P_H 5.26 and 12.5 cc. (NaOH), respectively, while the corresponding values for the stalks of the same plants were lower than the average concentration, being P_H 5.66 and 2.3 cc. Likewise, in Series V the hydrogen-ion concentration of the leaves of strain 150 seemed abnormally high with respect to the corresponding titratable-acidity measurement, but the acidity measurements of the stalks were much lower than those of any other strain in the plot. Moreover, in all the plants of every other strain in Series V the P_H value of the stalk was at least 0.2 below that of the leaves; yet in these diseased plants of strain 150 the figures were P_H 5.35 and 5.37, respectively.

DISCUSSION

Sufficient data have been presented to show that not only the titratable acidity, but also the hydrogen-ion concentration, of the expressed juice of corn plants is extremely variable. It has been shown that there exists a most striking dependence of acidity upon the reaction of the plant to its environment in so far as we can judge of this reaction by vegetative vigor. In Clevenger's (*6, p. 230*) article are discussed some of the ways in which he and others have found acid accumulation to be dependent upon internal and external factors.

It would not be amiss at this point to summarize the various P_H values reported in the literature for corn juice. Truog and Meacham (*16*) give 5.2 and 5.3 for the expressed juice of whole tops of corn plants from unlimed and limed soil, respectively. Haas (*8*) gives 5.19 and 5.48 for the tops of two different plantings, the first in Plainfield sand and the second in Colby silt loam. Bryan (*4*) found a variation from 5.1 to 5.2 in corn leaves, the plants being grown in sand and in solution cultures. Bauer and Haas (*3*), by applying various fertilizer treatments to quartz sand cultures, obtained values varying from 5.31 to 5.49 in leaves and from 5.31 to 5.95 in stalks.

The range of P_H and titratable acidity values for entire corn tops obtained in the present investigation is given in Table VI, together with the more obvious environmental factors and estimates of the relative vigor of the plants. These values are the averages from Tables I, II, III, IV, and are brought together to facilitate intercomparisons. Each value is an average of all the measurements made on the respective series, each composed of 40 to 50 plants, and including an approximately equal number of plants of each of the 10 strains. Both the maximum and the minimum measurements of titratable acidity obtained in each series and given under "range" follow the same order as do the respective means, and make the reality and significance of differences between the latter more convincing.

TABLE VI.—*Summary of the effect of environment on the titratable acidity and hydrogen-ion concentration of tops of corn plants and the apparent correlation of these characters with vegetative vigor*

Se-ries.	Months grown (1922).	Environment.				Relative vigor.	P_H.	Total acidity (cc. N/10 NaOH).		Specific gravity.
		Place.	Soil.	Soil reaction.	Outdoor temperature.[1]			Mean.	Range.	
				P_H	°C.					
I	April–May	Greenhouse A deep bench	Sandy loam	7.0	62	Very good	5.5	5.5	4.4–6.6	......
II	May–June	Greenhouse B shallow bench	Sandy clay loam	6.5	69	Poor	5.3	6.8	5.7–8.8	......
III[2]	May–July	...do	...do	6.2	73	Very poor	5.1	10.3	6.9–12.3	1.0233
IV	June–July	Greenhouse A deep bench	Sandy loam	7.2	76	Very good	5.5	4.7	3.7–5.6	1.0149

[1] Averages of hourly temperature readings of the Weather Bureau for each day during the growth of the series.

[2] Grown in the same soil as Series II, planted between the rows before that series was cut.

Every strain of corn from these different plantings showed variations in the acidity measurements parallel to those of the group averages of Table VI. To illustrate graphically a typical instance, the electrometric titration curves obtained with plants of a representative strain, No. 315, from the four greenhouse plantings, are plotted side by side in figure 2.

Whatever may be the cause of the differences in the vegetative vigor of these series and the correspondingly wide variation in their cell-sap acidity, temperature may be excluded except for Series V, by reason of the unrelated sequence of good and poor series. The illumination was probably nearly the same for all the series, especially for those grown within the same greenhouse. Since corn has been found (1, 3, 4, 9, 10, 14, 15, 17) to grow well in soils and nutrient solutions of even higher acidity than that of the poorest plot ($P_H6.2$), soil acidity can probably be eliminated as a causal factor. From the facts that the vigorous Series I and IV were grown in deeper soil than were Series II and III, and, that the
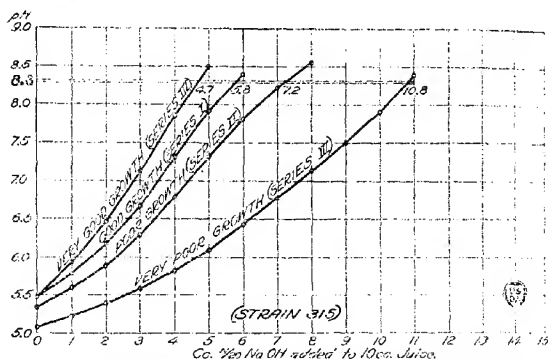

FIG. 2.—Electrometric titration curves of the juice of tops of Reid Yellow Dent corn from the four greenhouse plantings, illustrating the correlation of acidity measurements with vegetative vigor.

poorest series, III, was planted in relatively shallow soil which had already been partly exhausted by one crop (Series II) it would appear that the inadequacy of some soil constituents was responsible for the spindling growth and high acidity of Series II and especially of III. Series V evidently was stunted as a result of low temperatures, since it is common experience that the cool nights of early fall prevent good growth of corn.

Consistent with the inverse relation between vegetative vigor and acid accumulation brought out by intercomparisons of the series or of the strains composing them, are those individual measurements (Tables I, II, III, IV) which show a lower acidity of the more vigorous individuals of the same strain as compared to the poorer ones growing beside them. This was true not only of the leaf juice but also of that of the stalk, the latter showing the correlation the more strikingly. Occasional departures from the rule occurred and from these exceptions it is evident that neither green weight nor height are invariably indications of relative acidity. A short stalky plant was occasionally less acid than a tall more slender one which had been judged the better plant.

In general, there is no parallelism between the magnitude of the individual readings of acidity and specific gravity in the tables. However, certain striking instances of high sap density accompanying high acid content strongly suggest some interdependence. Some examples of this relation are the wilted plants of strain 485 in Series V (Table IV). These external symptoms of a water deficit were accompanied by an unexpectedly high acidity and high specific gravity of the juices. The plants of strain 972 (Series IV) were so wilted, as the result of not having been watered the day before, that the leaves were limp and somewhat rolled. The titratable acidity measurements of both leaf juice and stalk juice were higher than the corresponding values for the unwilted plants of the same strain, and their specific gravity values were abnormally high, 1.0317 for leaf juice and 1.0246 for stalk juice. Further indication of a possible relationship of these characters is found in the fact that leaf juices always had a definitely higher sap density than the less acid stalk of the same plant. However, there are so many instances of juices which differ widely in acidity yet show no significant difference in specific gravity that difference in the hydration of the tissues obviously is not the only factor determining the magnitude of the difference in acidity values.

No conclusions can be drawn from these experiments as to the existence of varietal differences in acidity because the individual plants of each strain varied greatly in growth and vigor, and consequently in acidity, even when growing side by side in the same series. The varieties showing the most pronounced vigor, such as 507 and 485, usually were among the least acid in each series, suggesting that growth reactions to the environment rather than inherent acidity characters were responsible for the acid differences. Moreover, the varieties did not keep the same relative positions as to vigor in the different series, some reacting more poorly in one environment, while being among the best in another, thus recalling the work of Mooers (12) who found great differences in the relative yields of varieties on different soils. It would seem, therefore, that environmental factors, together with the capacity of the strain to respond to them as indicated by its vigor of growth, are more potent in determining cell-sap acidity then preexisting varietal acid tendencies.

SUMMARY

(1) The hydrogen-ion concentration of the tops of corn plants ranged from P_H 5.0 to 5.6 in the five plantings constituting these experiments, and was inversely correlated with the degree of vegetative vigor induced by the environmental conditions affecting the different plots.

(2) The titratable acidity of these tops varied correspondingly, the values ranging from an average of 10 cc. of $N/20$ sodium hydroxid solution, required to neutralize 10 cc. of juice from plants of the most stunted plot, to 5 cc. (average) required to neutralize the same quantity of juice from the most rapidly growing plants.

(3) A lack of exact correlation between the magnitude of the acidity measurements and the specific gravity determinations of these juices shows that, in general, variations in sap density were not responsible for the variations in acid concentration. However, a number of striking instances of exceptionally high acidity values accompanied by correspondingly high density figures indicate that the water content of the tissues had, at times, a measurable effect on the acid concentration.

(4) The concentration of titratable acid was always higher in the juice of the leaves than in that of the stalk regardless of the plant's vigor. The hydrogen-ion concentration was higher in the leaves than in the stalk in the vigorous plants only; in the stunted plants it was greater in the stalks than in the leaves.

(5) In the slow-growing plants of the most stunted plot, the titratable acid concentration of the stalk was at least one-half that of the leaves; in the vigorous rapidly growing series this ratio was one-third or one-fourth and occasionally even lower.

(6) The specific gravity of the juice of the leaves was always higher than that of the juice of the stalk regardless of the plant's vigor.

(7) Environmental conditions produced far greater variations in the acidity of plants of the same strain than were ever found between plants of different varieties in the same environment and of equal vigor.

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